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SPECIFICATION

INOCULATING AGENT, SPAWNED INSECT AND METHOD OF PRODUCING FRUIT BODIES OF ENTOMOPATHOGENIC FUNGI

Technical Field

The present invention relates to an inoculating agent, a spawned insect and a production method for production of fruit bodies of entomopathogenic fungi, and more specifically it relates to an inoculating agent, a spawned insect and a production method for convenient mass production of fruit bodies of entomopathogenic fungi such as *Cordyceps*.

Background Art

Entomopathogenic fungi, such as those of *Cordyceps*, are used as materials for Chinese herbal medicines, high-grade foods and the like. However, natural sources are usually expensive since they must be collected in the field, and in many cases their quality is not uniform. Much is still unknown about the ecology of entomopathogenic fungi, and it is difficult to obtain fruit bodies of uniform quality by collection in the field. Efforts have therefore been made at artificial culturing of entomopathogenic fungi.

For example, there has been documented a method of obtaining fruit bodies whereby *Cordyceps* that have been artificially inoculated into parasitic insects are cultivated while controlling the host temperature (Japanese Patent Application Laid-open No. 8-75). Here, a suspension of

ascospores is used for the inoculation, and the inoculation is carried out by dipping the pupa in the suspension.

According to another document, silkworm pupa extract is obtained, an artificial medium containing it is prepared, and the medium is used to form a fruit body (Japanese Patent Application Laid-open No. 10-42691).

Attempts have also been made to dip the pupae in the ascospore suspension or inoculate hyphae by contact with the pupae, or to inject the pupae with the ascospore suspension; however, it has been documented that injection of ascospore suspensions does not give mature fruit bodies (Nippon Kingakukai Kaiho "Journal of the Mycological Society of Japan", 36:67-72, 1995, Harada et al.).

Disclosure of the Invention

As explained above, a number of attempts have been made to use spores such as ascospores or conidia, or hyphae for artificial culturing of *Cordyceps*, but the following problems have been presented in using ascospores, etc. as inocula.

Ascospores are problematic in that they are difficult to collect as inocula. Fruit body generation is extremely rare for entomopathogenic fungi such as *Cordyceps*, and hence there are very few opportunities which allow collection of ascospores from fruit bodies in the field. That is, it is very difficult to collect large amounts of ascospores in the wild to allow use of the ascospores as inocula. For example, it is said that outbreaks of *Cordyceps militaris* occurs in

mass every approximately 10 years in the Tohoku region of Japan, while in the other years almost none occurs, so that collection of ascospores is virtually impossible.

Even if they could be collected in the field, it would be difficult to collect the ascospores in large amounts and without contamination, for inoculation of numerous insects from only a few fruit bodies. Various types of bacteria adhere to the surfaces of fruit bodies growing in the field. This bacterial contamination cannot be avoided simply by cutting the acrospore-formed sections from the fruit bodies and collecting the acrospores separated from the fruit bodies as inocula. When a mixture of bacteria and *Cordyceps* ascospores is inoculated the bacteria usually proliferate faster, and therefore the host insect decays, thus preventing formation of fruit bodies.

When carrying out isolation culture from ascospores or fruit bodies on an agar medium such as Sabouraud's medium, *Cordyceps* usually grow in the form of hypha. However, because hyphae have a filamentous structure they tend to clog needles when injected for inoculation, and therefore hyphae are not suitable as inocula for injection.

A problem also exists with conidia (also called conidiospores) as inocula. Hyphae cultured on agar medium form conidia. These conidia can be used as inocula, but obtaining amounts suitable for mass inoculation is laborious. The following is a common method used to obtain conidia.

First, the hyphae are densely grown with agar medium in a

petri dish, and conidia are formed thereon. Sterilized distilled water or the like is poured into the petri dish and the hyphae are scraped with a sterilized glass rod to pull the conidia from the hyphae. The conidia suspension obtained in this manner can be used as an inoculum, but it is necessary to repeat the operation of pouring sterilized water into each petri dish and scraping to obtain the conidia suspension. While 10-20 ml of sterilized water is used for each petri dish, most of the conidia remain in the petri dish even after scraping, and this high loss of the inoculum results in poor efficiency.

Conidiogenesis begins on about the second day after the hyphae are inoculated in the agar medium (25° C, 24-hour illumination). When the formed conidia have been inoculated on the agar medium, the germination rate of the conidia is 90% for conidia on the third day after hypha inoculation but falls to 50% by the ninth day after hypha inoculation, and the germination rate decreases rapidly thereafter to virtually zero germination by the 15th day after hypha inoculation. The fungus colonies grow in a concentrical fashion and therefore the oldest conidiospores are at the center. When conidia are used as inocula, the older conidia and newer conidia are mixed and therefore the inoculum has a non-uniform germination rate, so that after inoculation it is difficult to achieve stability of insect mortality and forming rate of fruitbodies, for example. In other words, it is difficult to obtain large amounts of conidia with stable properties.

In methods of percutaneous infection by inoculation of fruit bodies, hyphae or conidia onto the body surfaces of insects, it requires 75-100 days or more in the case of *Cordyceps militaris*, for example.

It is an object of the present invention, which has been accomplished in light of the problems described above, to achieve convenient, rapid, economical mass production of fruit bodies of entomopathogenic fungi such as *Cordyceps* with uniform quality.

As a result of much diligent research toward this aim, the present inventors have completed the present invention upon finding that fruit bodies of entomopathogenic fungi can be easily produced in mass by using hyphal bodies of entomopathogenic fungi.

Specifically, the present invention is as follows.

(1) An inoculating agent for production of fruit bodies of an entomopathogenic fungus, containing hyphal bodies of the entomopathogenic fungus.

(2) A method of producing an inoculating agent for production of fruit bodies of an entomopathogenic fungus, whereby hyphal bodies are produced by shake culturing of hyphae and/or conidia of the entomopathogenic fungus.

(3) A method of producing an inoculating agent for production of fruit bodies of an entomopathogenic fungus, whereby hyphal bodies are propagated by shake culturing of hyphal bodies of the entomopathogenic fungus.

(4) A spawned insect for production of fruit bodies of an

entomopathogenic fungus, being inoculated with hyphal bodies of the entomopathogenic fungus in the body.

(5) A method of producing fruit bodies of an entomopathogenic fungus, whereby hyphal bodies of the entomopathogenic fungus are inoculated into a body of an insect.

(6) A method of producing fruit bodies of an entomopathogenic fungus described above, wherein said inoculation is accomplished by injecting.

(7) A method of producing fruit bodies of an entomopathogenic fungus described above, wherein said insect is in a form of a pupa.

The present invention also relates to the method of producing fruit bodies of an entomopathogenic fungus described above, wherein said entomopathogenic fungus is a fungus belonging to the genus *Cordyceps*.

While not all aspects of the life cycle of entomopathogenic fungi are understood, a general overview of the life cycle is shown in Fig. 1. The life cycle is thought to be generally as follows, using *Cordyceps militaris* which is an entomopathogenic fungus and a kind of *Cordyceps* spp. as an example.

The fruit body of *Cordyceps militaris* is a bundle of hyphae, with a portion of the tip of the fruit body forming a clavate stroma. The stroma includes a collection of numerous half-buried fine granules, which are known as the perithecia.

Numerous asci appear when the perithecium is broken, and these asci contain filamentous spores. The spores are reproductive cells that on their own can become new individuals of the plant or fungus, and the spores in the asci are known as ascospores. The ascospores are sexual spores. The ascospores leaving the perithecia to the outside will germinate in certain favorable environments such as under fallen leaves, and form hyphae consisting of multiple cells. Asexual spores known as conidia sometimes form from some parts of the hyphae. In the natural environment, the hyphae as a rule penetrate through body surface of insects and infect in the soil, for example pupal stage insects. Hyphae that have invaded the body of an insect by percutaneous infection produce hyphal bodies that display a yeast-like form, and proliferate throughout the body. Progressive proliferation in the body results in death of the insect, while the hyphal bodies become the form of hyphae again, and the hyphae bundle together and exit the body of the host to form fruit bodies.

The terms used in this specification will now be explained. Throughout this specification, "entomopathogenic fungi" is a general term referring to eukaryotes such as mushrooms, fungi, yeasts and Myxomycetes that form fruit bodies and of which hosts are insects, but not including prokaryotic bacteria. And, "fruit body" used in this specification refers to a hyphal bundle which has formed a stroma or synnema. When only fruit body is mentioned, it includes both hyphal bundles grown from asexual spores and hyphal bundles grown from sexual

spores.

In addition, "*Cordyceps*" used throughout the present specification will refer to fungi belonging to the subphylum Ascomycotina, class Pyrenomycetes, order Clavicipitales, family Clavicipitaceae, genus *Cordyceps*, and it is included in entomopathogenic fungi.

Moreover, "hyphal body" used in the specification refers to cells in the form that appears when the entomopathogenic fungi proliferate in the bodies of insects. A hyphal body usually consists of one cell which appears morphologically in a yeast-like form and is thus distinguished from a hypha. A yeast-like form is the condition of proliferation in a unicellular cylindrical shape by budding. The form in which the entomopathogenic fungus appears when proliferating in the body of an insect is sometimes referred to as a blastospore, cylindrical spore, short hypha, segmented cell and the like, but throughout this specification all the forms in which entomopathogenic fungi appear when proliferating in the bodies of insects will be referred to as hyphal bodies.

A "spawned insect" as used in this specification is an insect that is a host for fungi and serves as a site of growth for the fungi.

According to the present invention there are provided an inoculating agent and spawned insects that produce fruit bodies of prescribed entomopathogenic fungi, and they may be used for convenient mass production of fruit bodies of the entomopathogenic fungi.

The invention will now be explained in greater detail.

<1> Inoculating agent of the present invention

The inoculating agent of the present invention will be explained first. The inoculating agent of the present invention is characterized by containing hyphal bodies of an entomopathogenic fungus. The entomopathogenic fungus is one such as described above. The present invention may be applied to any entomopathogenic fungi that can form hyphal bodies, and examples of such fungi are those belonging to the genera *Cordyceps, Akanthomyces, Beauveria, Gibellula, Hirsutella, Hymenostilbe, Metarhizium, Nomuraea, Paecilomyces, Paraisaria, Stilbella, Tilachlidium* and *Tolypocladium*, among which fungi of *Cordyceps* are more preferred, and *Cordyceps sinensis* and *Cordyceps militaris* are especially preferred.

Hyphal bodies are described above. An inoculating agent containing hyphal bodies can be obtained by shake culturing the hyphae and/or conidia of the entomopathogenic fungi. Shake culturing of the hyphae and the like allows easy mass production of the hyphal bodies. Once the hyphal bodies are obtained, the shake culturing may be carried out from the hyphal bodies for propagation of the hyphal bodies.

The medium used for the shake culturing is usually a liquid medium from the standpoint of ease of handling. While the medium may be appropriately prepared depending on the type of entomopathogenic fungus, preferably Sabouraud's medium, potato extract broth, silkworm pupa extract broth or the like

are exemplified, and most preferably Sabouraud's medium or silkworm pupa extract broth are exemplified. Sabouraud's medium is an agar medium containing agar (Sabouraud glucose agar medium), but for shake culturing for the purpose of hyphal body propagation it is common to use a liquid medium without the agar. ("Sabouraud's glucose agar medium" is described in "Kinruizukan (Atlas of Fungi), p.1279, No.25, Kodansha".) Sucrose, yeast extract and the like may also be combined with the above-mentioned liquid medium. As a particularly preferred example of a liquid medium there may be mentioned yeast extract-added Sabouraud's sucrose liquid medium, wherein the sugar of Sabouraud's medium is changed from glucose to sucrose, and yeast extract is added.

While it is sufficient for the inoculating agent of the invention to include the hyphal bodies, an inoculating agent according to the present invention may also be prepared, for example, by diluting the hyphal bodies with the liquid medium in which the hyphal bodies have proliferated, as explained above, to adjust the hyphal bodies to an appropriate concentration. For example, in the case of an inoculating agent such as an injection, a biologically acceptable solution may be used as the liquid solution containing the hyphal bodies, and specifically the liquid medium in which the hyphal bodies have proliferated may be used directly, or a liquid such as sterilized distilled water, liquid medium for insect cell culture, physiological saline, Carlson's fluid or the like may be used. The inoculating agent of the invention may

also include other components that are included in inoculating agents for inoculation into insect bodies.

While the conditions such as the temperature and degree of shaking during the liquid culturing for propagation of the hyphal bodies may be appropriately adjusted depending on the type of entomopathogenic fungus, preferred conditions are the following. The preferred temperature for liquid culturing is 20-25°C. The degree of shaking may be adjusted as appropriate, and for example, shaking at about 100-110 (oscillations/minute) is sufficient. The shaking culture to obtain the hyphal bodies is preferably carried out in the dark.

The conditions for culturing of the hyphal bodies may be the same whether the culturing is initiated from hyphae, conidia or whether the culturing is initiated from the hyphal bodies themselves that have already been obtained.

Hyphal bodies obtained under the conditions described above are synchronized and uniform, and this contributes to the stable quality of the inoculating agent. Here, "synchronized" means having the same growth rate, and synchronization gives a large amount of hyphal bodies in the same growth stage.

While there are no particular restrictions on the form of the inoculating agent of the present invention, it is preferably a form that can be directly introduced into insect bodies, with injections being specifically preferred. As will be explained below, an injection can give a higher infection rate and contribute to stable production of fruit bodies. A

suspension containing the hyphal bodies cultured in liquid medium may also be prepared to a suitable density for direct use as an injection.

<2> Spawned insect of the present invention

The spawned insect of the present invention will now be explained. The spawned insect according to the present invention is characterized by being directly inoculated with hyphal bodies of entomopathogenic fungi into the body, whereby the hyphal bodies are retained in the body.

While the insects to be prepared as the spawned insects will depend on the type of entomopathogenic fungi to be inoculated, preferred ones include *Mamestra brassicae*, *Tenebrio molitor* (also known as mealworm), *Bombyx mori*, *Galleria mellonella* and *Spodoptera litura* and the like, among which particularly preferred ones are *Mamestra brassicae*, *Tenebrio molitor* and *Bombyx mori* and the like. However, the insect to be prepared as the spawned insect may be selected without any limitation to naturally occurring combinations of hosts and entomopathogenic fungi, and insect other than existing host may be used as the spawned insect.

The insect to be prepared as the spawned insect is suitably used in the form of pupa, because they require no feeding and can be packed at a high density. The insect to be prepared as spawned insect may be imago or larva, and they may be living individual or non-decayed dead one, and they need not necessarily be in diapause. For example, frozen pupa or

the like may also be used.

The inoculation of the hyphal bodies into the insect bodies may be accomplished by a method such as injecting into the insect using an inoculating agent of the present invention as described above in the form of an injection. After the hyphal bodies have been introduced into the insect, they may be placed under conditions suitable for fruit body formation, to form the fruit bodies. The spawned insect of the present invention may be stored for over a month controlled at low temperature (about 4-5°C), though this will depend on the types of inoculated entomopathogenic fungus and host insect.

<3> Fruit body production method of the present invention

A method of producing fruit bodies of entomopathogenic fungi according to the present invention will now be explained. The fruit body production method of the present invention is characterized by inoculating hyphal bodies of an entomopathogenic fungus in the body of an insect. According to this method of the present invention, it can be accomplished by introducing the hyphal bodies of the entomopathogenic fungus directly into the insect body, and for example, it can be accomplished by injecting the host insect with the inoculating agent of the present invention described above in the form of an injection. Inoculation of insect bodies with injections contributes to a higher infection rate, and more stable and rapid production of fruit bodies. When the inoculating agent of the present invention is injected

into the insect body, the concentration of the hyphal body in the inoculating agent is preferably 10^4-10^7 cells/ml, and especially 10^6-10^7 cells/ml, though it will depend on the type of entomopathogenic fungus. A concentration within this range is preferred from the standpoint of increasing the infection rate and shortening the period from inoculation to formation of fruit bodies. A concentration within this range also results in fewer working problems such as injection needle clogging.

The inoculation dose of the hyphal bodies can be appropriately adjusted based on the size of the insect and the concentration of the inoculating agent. It is normally an inoculation of the maximum amount of hyphal bodies, but at a dose such that the inoculating agent does not flow out of the insect body. A greater amount of hyphal bodies introduced into the body will tend to result in earlier death of the host insect, thus shortening the period to formation of fruit bodies.

The preferred types and forms of the insects to be inoculated with the hyphal bodies are the same as those mentioned above as preferred for the spawned insect of the present invention.

The types of entomopathogenic fungi preferably used for the fruit body production method of the invention are the same entomopathogenic fungi as those mentioned above as preferred for the inoculating agent of the present invention.

The fruit bodies may be produced from the insects by

keeping the hyphal body-inoculated insects (i.e. the spawned insects) under environmental conditions of suitable temperature, humidity, etc. These will depend on the type of inoculated fungus and the type of host insect, but as an example of preferred conditions there may be mentioned the following. A temperature of 20-25° C is preferred for formation and growth of fruit bodies from insects inoculated with the hyphal bodies. Within this range it is possible to shorten the period until formation of the fruit bodies. Below this range, formation of fruit bodies is often difficult or the period of fruit body formation is prolonged. The spawned insects are preferably kept in humid conditions, specifically a humidity of 90-100%. The spawned insects can be easily controlled to such temperature and humidity conditions by using sphagnum moss. The illumination is preferably 50-350 lx, and the lighting period length may be adjusted as appropriate.

<4> Advantages of using hyphal bodies

The major advantage of using hyphal bodies as the inoculating agent is that mass production can be achieved within a shorter time. Since hyphal bodies can be cultured in liquid medium, they can be immediately used as an injection by simply adjusting the hyphal body-containing culture medium to an appropriate concentration, and handling is also facilitated.

For example, in order to obtain conidia in the same amount as a single growth culture of hyphal bodies in 200 ml of liquid medium using a 500 ml flask (25° C, yeast extract-added

Sabouraud's sucrose liquid medium), it is necessary to plant the hyphae in 10-20 petri dishes, add sterilized distilled water, and then scrape and prepare a suspension, all of which requires considerable labor to be carried out.

The hyphal bodies are vegetative cells of the entomopathogenic fungi that appear in the body of insects in the natural world. According to the present invention, the insects can be killed within a short period by forced introduction of the previously prepared vegetative cells into the bodies of the insects. In the field, it is thought that proliferation begins by percutaneous infection when the *Cordyceps* hyphae or conidia attach onto the body surface of the insects, and this process is said to normally require about 40 days to result in death of the pupal insect, in the case of *Cordyceps militaris*. However, injecting of hyphal bodies can kill the insects in 2-3 days. The shorter time period until death of the insects is thought to be brought about because a high concentration of hyphal bodies are injected directly into the blood of the insects. This drastic reduction in the time from inoculation to fruit body formation constitutes a major advantage of hyphal bodies as inocula.

Furthermore, the hyphal bodies can also be stored for over a month if controlled to low temperature (about 5°C) in a liquid medium suspension state. Hyphal bodies are also a convenient inoculum from this standpoint of storage efficacy.

Hyphal bodies can be propagated in mass with uniform quality, allowing efficient production of fruit bodies. Once

the ascospores, hyphae, etc. have been obtained, the hyphal bodies can be inexpensively grown thereafter.

Brief Description of the Drawings

Fig. 1 is a diagram showing a summary of the life cycle of entomopathogenic fungi.

Best Mode for Carrying Out the Invention

The present invention will now be explained in further detail by way of examples, with the understanding that the invention is in no way limited to these examples.

<Example 1> Preparation of inoculating agent

An inoculating agent containing *Cordyceps militaris* hyphal bodies was prepared in the following manner. The *Cordyceps militaris* used was obtained from Mt. Iwaki (Aomori Prefecture) in Japan in July of 1995, and was kept on agar medium at the Forestry and Forest Products Research Institute of the Forestry Agency of the Ministry of Agriculture, Forestry and Fisheries (Forestry and Forest Products Research Institute, Forest Biology Division, Insect Pathology Laboratory: Strain No. F-1176-21). Hyphae were obtained from preserved culture of *Cordyceps militaris*, and the hyphae were planted to yeast extract-added Sabouraud's sucrose liquid medium for culturing for 5-7 days by a liquid shake culturing method (25°C, in the dark). The yeast extract-added Sabouraud's sucrose liquid medium had the composition shown in Table 1, with peptone,

yeast extract and sucrose added to water. The yeast extract used was BACTO[R] YEAST EXTRACT by DIFCO Co.

After 7 days, the hyphal bodies of *Cordyceps militaris* in the liquid medium were confirmed to have grown to approximately 10^7 cells/ml.

The concentration of hyphal bodies in the liquid medium was adjusted to prepare inoculating agents to 2.1×10^7 cells/ml, 1.7×10^7 cells/ml and 2.3×10^7 cells/ml.

<Table 1> Composition of yeast extract-added Sabouraud's sucrose liquid medium (pH: 6.5)

Component	Content
Peptone	10 g
Yeast extract	10 g
Sucrose	20 g
Distilled water	1 liter

<Example 2> Production of fruit bodies

(Example 2-1)

Mamestra brassicae pupae were injected with an inoculating agent containing hyphal bodies, and fruit bodies were formed under three different temperature conditions. The specific procedure was as follows.

The inoculating agent with a hyphal body concentration of 2.1 x 10^7 cells/ml prepared in the above mentioned Example 1 was injected at 5 µl per a *Mamestra brassicae* pupa with an improved microdispenser having a thinned tube and a sharpened tip. The *Mamestra brassicae* pupae inoculated with the hyphal bodies were incubated while buried in sphagnum moss moistened with water. After inoculating 90 pupae, 30 each were kept under controlled temperature conditions of 15°C, 20°C and 25°C, respectively. The illumination was 100-300 lx at all the temperatures, with a 14 hour light period and a 10 hour dark period. The details of the conditions up to the 47th day after inoculation are shown in Table 2.

Table 2 Results for Example 2-1

Temperature	Number of insects injected	Development stage			Days		
		Appearance of young fruit bodies	Appearance of fruit bodies with perithecia		Appearance of young fruit body appearance	Appearance of fruit bodies with perithecia	
			Initial	Matured		Initial	Matured
25°C	30	30	29	29	21 days	30 days	34 days
20°C	30	30	15	1	21 days	43 days	46 days
15°C	30	0	0	0	-	-	-

As shown in Table 1, a minimum of 34 days was required for maturation of the fruit bodies at 25°C, a minimum of 46 days was required at 20°C, and no fruit bodies were obtained at 15°C.

In a previous report using *Mamestra brassicae* (Nippon Kingakukai Kaiho "Journal of Mycological Society of Japan", 36:67-72, 1995, Harada et al.), at least 75 days were required after inoculation to form mature fruit bodies, but according to the invention it was possible to obtain mature fruit bodies at least one month earlier.

(Example 2-2)

Tenebrio molitor pupae were injected with an inoculating agent containing hyphal bodies, and fruit bodies were formed under three different temperature conditions, in the same manner as Example 2-1. The inoculating agent used had a hyphal body concentration of 1.7×10^7 cells/ml, and a total of 135 insects were injected, with 45 each kept at the different temperatures. The other conditions were the same as in Example 2-1. The details of the conditions up to the 47th day after inoculation are shown in Table 3.

Table 3 Results for Example 2-2

Temperature	Number of insects injected	Development stage			Days		
		Appearance of young fruit bodies	Appearance of fruit bodies with perithecia		Appearance of young fruit body appearance	Appearance of fruit bodies with perithecia	
			Initial	Matured		Initial	Matured
25°C	45	32	12	7	19 days	26 days	29 days
20°C	45	20	4	4	19 days	32 days	40 days
15°C	45	5	0	0	26 days	-	-

Tenebrio molitor belongs to the order Coleoptera, which is taxonomically very different from *Mamestra brassicae* belonging to the order Lepidoptera, and it is not a natural host for *Cordyceps militaris*. It has been demonstrated, however, that according to the method of the invention it is possible to form fruit bodies using *Tenebrio molitor* which is normally not a host in the natural environment. It has also been demonstrated that fruit body formation of *Cordyceps militaris* can be achieved in a shorter time even when *Tenebrio molitor* is used as the host.

(Example 2-3)

Bombyx mori pupae were injected with an inoculating agent having a hyphal body concentration of 2.3 x 10^7 cells/ml at 100 µl per insect, and the pupae were incubated while buried in sphagnum moss moistened with water and kept in a laboratory at 25°C room temperature. Formation of mature fruit bodies was confirmed by the 47th day at the earliest.

Because *Bombyx mori* pupae are larger than the pupae of the insects used in Examples 2-1 and 2-2, they can be easily inoculated with a syringe, such as a human tuberculin syringe,

thus allowing more convenient production of fruit bodies. Sterilized human tuberculin syringes are readily available, and while some experience is necessary for the inoculation procedure using a microdispenser as described in Example 2-1, even a novice can easily perform inoculation procedure using a human tuberculin syringe.

The *Bombyx mori* pupae are not in diapause, and therefore it was demonstrated that the method of the invention allows formation of fruit bodies even from insects that are not in diapause.

Industrial Applicability

According to the present invention it is possible to mass-produce inoculating agents conveniently and inexpensively for production of fruit bodies of entomopathogenic fungi such as *Cordyceps*. An inoculating agent of the present invention can be produced with uniform quality, it is easy to manage, and it can also be stored. The present invention also allows convenient, rapid, inexpensive and efficient mass production of fruit bodies of entomopathogenic fungi such as *Cordyceps* using this inoculating agent and so on. The invention also makes it possible to produce fruit bodies regardless of the season.

Entomopathogenic fungi such as *Cordyceps* can be used as materials for Chinese herbal medicines, high-grade foods and the like. Entomopathogenic fungi are also being developed for use as biological pesticides. The present invention is

expected to contribute to stabilized mass supply of fruit bodies of entomopathogenic fungi, for fields that utilize those entomopathogenic fungi.